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Comparative analysis of the structure of the allopolyploid liverwort *Pellia borealis* and ancestral taxa

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Abstract: *Pellia borealis* Lorb. is a simple thalloid liverwort that originated after hybridization between two allopatric taxa: *Pellia epiphylla* N *and Pellia epiphylla* S. The morphological and anatomical similarity of *Pellia* species along with its plasticity cause difficulties in clearly defining the species. Species from the *Pellia epiphylla* complex differ from the remaining *Pellia* species by bisexuality. Microstructure of the gametophytes of all three taxa and sporophytes of *P. borealis* and *P. epiphylla* S was investigated using scanning electron microscopy. As a result of these observations, some new diagnostic characters between analyzed taxa were discovered. *P. borealis* shows a different pattern of papillae distribution on the dorsal surface of the thallus compared to ancestral taxa and has larger cells. *P. epiphylla* N was distinguished by the localization and shape of archegonia. Comparison of *P. borealis* and *P. epiphylla* S capsule surface showed a significantly different microstructure.

Key words: liverworts, scanning microscopy, gametophyte, sporophyte

1. Introduction

The allopolyploid origin of *Pellia borealis* Lorb. is well documented by molecular analysis and *P. epiphylla* N and *P. epiphylla* S are identified as the parental taxa (Odrzykoski *et al.* 1996; Fiedorow & Szweykowska-Kulińska 1998). *P. borealis* is widespread in Poland (Szweykowski *et al.* 1995), and it was also found in the British Isles (Newton 1985) and in the Ardennes (de Sloover & Messe 1982), but it is not known beyond Europe. The ancestral taxa differ in geographic distribution in Poland – the first occurs in northern Poland and the second in the South (Szweykowski *et al.* 1995). *P. epiphylla* N and *P. epiphylla* S were recognized as a sibling species on the basis of isoenzyme (Zieliński 1987) and DNA markers (Pacak *et al.* 1998; Fiedorow *et al.* 2001). However, its morphological similarity did not allow it to be distinguished as a formal species and it is not known which of these two taxa refers to the *P. epiphylla* (L.) Corda. Difficulties in the proper identification of species from the *P. epiphylla* complex require a search for new diagnostic features at the level of microstructure. Thus the micromorphological structure of gametophyte and sporophyte was studied with scanning electron microscopy.

2. Material and methods

Analyses were conducted on gametophytes of *P. borealis, P. epiphylla* N and *P. epiphylla* S and on sporophytes of *P. borealis* and *P. epiphylla* S. Gametophytes of liverworts were obtained from the Department of Gene Expression, at Adam Mickiewicz University in Poznań, where they were introduced into an *in vitro* culture. A further culture was continued at the Department of Plant Anatomy and Cytology, University of Silesia. Sporophytes were collected from natural populations at the following stations: *P. borealis* – Diabli Skok Reserve near Wałcz, on the banks of the stream; *P. epiphylla* S – Tatra National Park, Zakopane, by the trail to Nosal Pass.

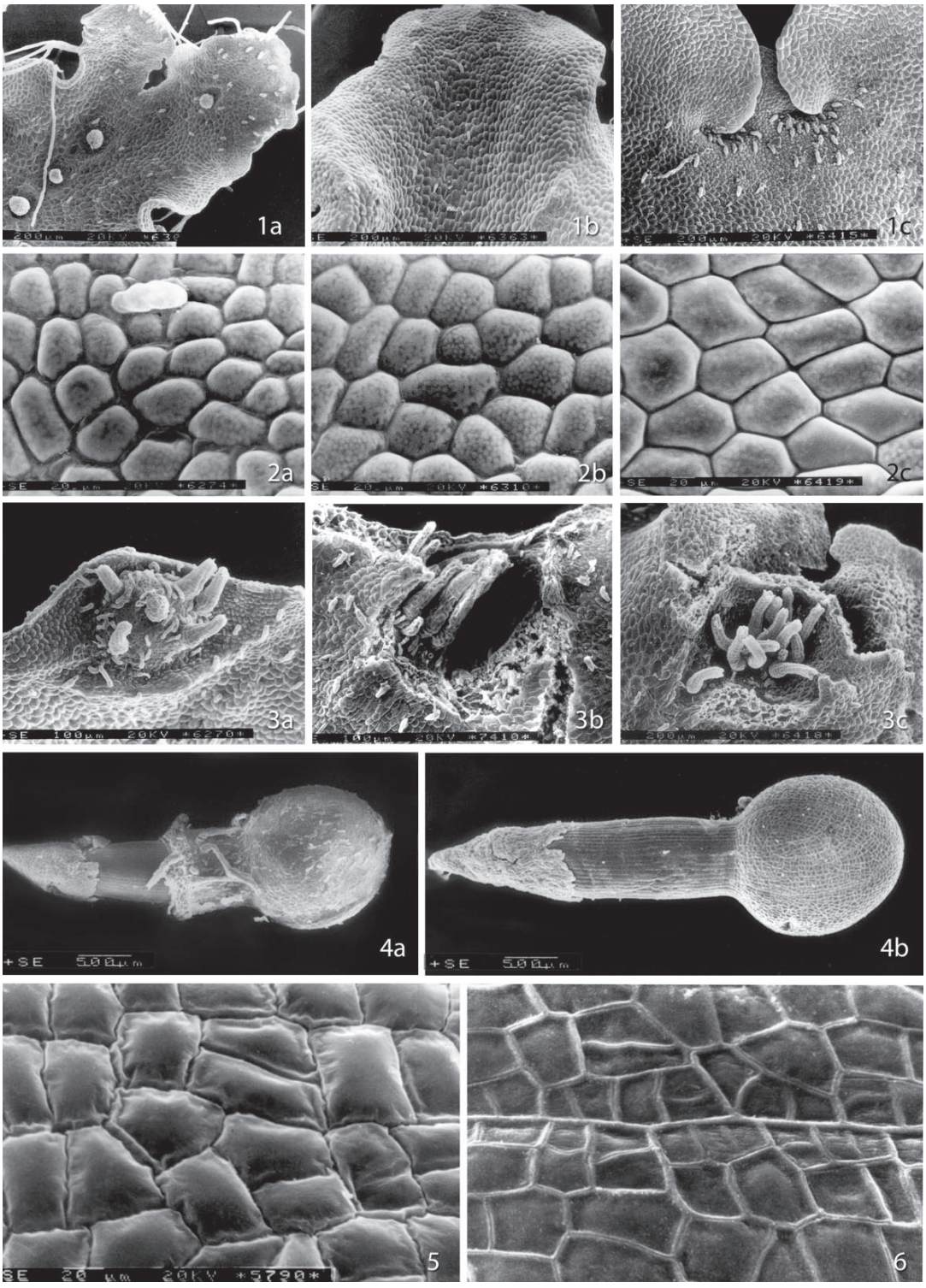
Fragments of gametophyte thallus and isolated sporophytes were fixed in a 3% glutaraldehyde solution and prepared for SEM analysis according to the Młodzianowski & Woźny (1981) protocol. Dehydrated material was critical point dried in liquid carbon dioxide (Pelco CPD-2), and coated with gold in a sputter coater (Pelco SC-6). Observations were performed with scanning electron microscope, Tesla BS 340, operating at 20 kV.

Microphotographs were taken using films FOMAPAN 400/120. Photographs were scanned with AGFA

SnapScan 1236 scanner. Graphic files were processed in Picture Publisher and Adobe Photo Shop software.

3. Results and conclusions

Gametophyte is the main generation in the liverwort life cycle. In the *Pellia* genus it has a dichotomously branched thallus with rhizoids on its ventral side and male and female reproductive organs on the dorsal side. SEM analysis showed that in *P. borealis* and its ancestors, the thallus surface consists of tetra-to heptagonal cells with convex outer periclinal walls and straight, slightly depressed, anticlinal walls. Some structural characteristics discriminating the polyploid species from its ancestors



Figs. 1-6. Microstructure of gametophyte and sporophyte in *Pellia epiphylla – Pellia borealis* complex
Explanations: 1 a-c – distribution of papillose structures on thallus dorsal surface; 2 a-c – microstructure of thallus dorsal surface; 3 a-c – archegonia structure (on Fig. 1, 2 and 3: a – *P. epiphylla* N; b – *P. epiphylla* S; c – *P. borealis*); 4 – *P. borealis* isolated sporophyte, a – with calyptra; b – without calyptra; 5 – microstructure of *P. borealis* capsule surface; 6 – microstructure of *P. epiphylla* S capsule surface

have been found. Firstly in the parental taxa, papillose structures, present at the thallus surface, are distributed irregularly on the entire dorsal surface while in the allodiploid they are mostly localized on the thallus's apical area between its folds (Figs. 1 a-c). Secondly, in the *Pellia borealis,* cells of the superficial thallus layer are bigger with narrower anticlinal boundaries than in the ancestral taxa (Figs. 2 a-c). SEM observations of the reproductive organs showed that there are no significant differences in the antheridia structure between the analyzed species. In contrast, the archegonia of the *P. epiphylla* N usually are localized on the thallus hill without pseudoperianth and they are bottle-shaped (Fig. 3 a), whereas in *P. borealis* and *P. epiphylla* S archegonia are more elongated and covered with pseudoperianth (Figs. 3 b-c).

Sporophyte of liverworts is coated with calyptra (Fig. 4 a) and consists of a foot, which forms a placental zone with the gametophyte, a seta and a capsule (Fig. 4 b). Most taxonomically informative structural characters concern the capsule wall. The thickenings of secondary cell walls within the capsule wall are a diagnostic feature which has been used for years in liverwort taxonomy (Crandal-Stotler & Stotler 2000). SEM observations indicated that the capsule surface cell patterns also can be used as good characteristics identifying the discussed taxa. In liverworts from the *Pellia* genus, the capsule wall consists of two cell layers. In *P. borealis,* cells of outer layer are tetra-to pentagonal in shape with clearly stressed angles. The outer periclinal cell walls are convex, whereas the anticlinal cell walls are slightly wrinkled and collapsed (Fig. 5). The capsule opens with four dehiscence sutures formed by two rows of smaller tetragonal cells. The outer layer of the *P. epiphylla* S capsule also has tetra-to pentagonal cells with depressed outer periclinal walls and straight, raised anticlinal cell walls. The capsule opens with four dehiscence sutures, as in the *P. borealis*, that are visible as a regular hill of anticlinal cell boundaries (Fig. 6).

These results revealed information about new diagnostic features of gametophyte and sporophyte structure in the *Pellia* genus. Described surface features can be used as good diagnostic characteristics for these very similar taxa. Observed differences between taxa confirm the molecular analyses and are another indication to distinguish cryptic ancestral taxa as distinct species.

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